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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
STAPLES, MARK	

ART UNIT	PAPER NUMBER
1637	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/729,581	KEEFE ET AL.	
	Examiner	Art Unit	
	MARK STAPLES	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *11 June 2009*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *See Continuation Sheet* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *See Continuation Sheet* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

Continuation of Disposition of Claims: Claims pending in the application are 1,5-12,14-17,19,20,77-79,81-85,88,90-94,101-110,112-114,116-120,122-127,130-137,139-148,150-159,161-174,176-190,195 and 196.

Continuation of Disposition of Claims: Claims rejected are 1,5-12,14-17,19,20,77-79,81-85,88,90-94,101-110,112-114,116-120,122-127,130-137,139-148,150-159,161-174,176-190,195 and 196.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 06/11/2009 has been entered.

2. Applicant's amendment of claims 1, 17, 81, 90, 101,102, 110, 122, 130-132, 137, 139, 148, 150, 159, 161,174 and 182; the cancellation of claims 18, 80, 89, 111,121, 138, 149, 160, 175, and 191-194; and the addition of new claims 195 and 196 in the paper filed on 06/11/2009 is acknowledged.

Claims 1, 5-12, 14-17, 19-20, 77-79, 81-85, 88, 90-94, 101-110, 112-114, 116-120, 122-127, 130-137, 139-148, 150-159, 161-174, 176-190, 195 and 196 are pending and at issue.

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Rejections that are Withdrawn

Canceled Claim Rejections Moot / Withdrawn

3. The rejections of canceled claims 18, 80, 89, 111,121, 138, 149, 160, 175, and 191-194 are moot and therefore are withdrawn.

Claim Rejections Withdrawn - 35 USC § 112 Second Paragraph

4. The rejections of claims 1, 5-12, 14-17, 19-20, 77-79, 81-85, 88-94, 101-110, 112-114, 116-127, 130-17, 139-148, 151-159, 161-174, 176-194 under 35 U.S.C. 112, second paragraph, as being indefinite are withdrawn as Applicant has made amendments to the claims to overcome the rejections.

Declaration under 37 C.F.R. § 1.132

5. Applicant's clarification of the graph in Exhibit A of the previously filed Declaration is acknowledged. However neither this clarification nor Applicant's further arguments overcome the new rejections in view of Chow et al. (1971) and Padilla et al. (December 15, 2002) as given below.

Claim Rejections Withdrawn - 35 USC § 103(a)

6. The rejection of claims 1, 5-12, 14-17, 19-20, 77-79, 81-85, 88-94, 101-110, 112-114, 116-120, 122-127, 130-159, 161-174, and 176-194 under 35 U.S.C. 103(a) as being unpatentable over by Pieken et al. (U.S. Patent 5,660,985 previously cited), Brieba et al. (Biochemistry (2000) 39:919-923 previously cited), Sousa et al (U.S. Patent

6,107,037 previously cited), and Bishop et al. (1971) is withdrawn. Applicant's arguments have been considered but are moot in view of the new ground(s) of rejection.

7. The rejection of claims 18, 80, 89, 111, 121, 138, 149, 160, and 175 under 35 U.S.C. 103(a) as being unpatentable over Pieken et al. (U.S. Patent 5,660,985), Brieba et al. (2000), Sousa et al (U.S. Patent 6,107,037) and Bishop et al. (1971) in view of Milligan et al. (Methods Enzymol. (1989) previously cited) is withdrawn. Applicant's arguments have been considered but are moot in view of the new ground(s) of rejection.

New Objections and Rejections

Specification

8. The abstract of the disclosure is objected to because paragraph 00115 discloses the "Y693F" single mutant when it appears that the "Y639F" single mutant is intended . Correction is required in this paragraph and throughout the specification as necessary. See MPEP § 608.01(b).

Priority

9. The later-filed application must be an application for a patent for an invention which is also disclosed in the prior application (the parent or original nonprovisional application or provisional application). The disclosure of the invention in the parent application and in the later-filed application must be sufficient to comply with the requirements of the first paragraph of 35 U.S.C. 112. See *Transco Products, Inc. v. Performance Contracting, Inc.*, 38 F.3d 551, 32 USPQ2d 1077 (Fed. Cir. 1994).

The disclosure of the prior-filed application, Provisional Application No. 60/430,761, fails to provide adequate support or enablement in the manner provided by the first paragraph of 35 U.S.C. 112 for one or more claims of this application. The prior application does provide support for the instantly claimed Y639F/H784A double mutant RNA polymerase and does not provide support for the use of the instantly claimed Y639F/H784A double mutant RNA polymerase for identifying apatmers, especially for incorporating 2'-OMe substituted NTPs and more specifically 2'-OMe substituted GTPs. Accordingly claims 8, 106, and 157 are not entitled to the benefit of the prior application.

The earliest priority date for claims 8, 106, and 157 is 07/15/2003 (July 15, 2003) which is the filing date of Provisional Application 60/487,474.

New Claim Rejections - 35 USC § 112, First Paragraph

10. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

11. Claims 6, 7, 104, and 105 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. There is no written support for the modified T7 RNA polymerases of these claims which are the Y639F mutant and the H784A mutant to incorporate 2'-OMe substituted GTP into a single nucleic acid as

recited in claim 1, 101, and 102. The instant specification expressly teaches that the the Y639F mutant does not incorporate 2'-OMe substituted GTP and does not provide support that the the H784A mutant does incorporate 2'-OMe substituted GTP :

"Generally, it has been found that under the conditions disclosed herein, the Y693F [sic, Y639F] single mutant can be used for the incorporation of all 2'-OMe substituted NTPs except GTP and the Y639F/H784A double mutant can be used for the incorporation of all 2'-OMe substituted NTPs including GTP. It is expected that the H784A single mutant possesses similar properties when used under the conditions disclosed herein" (see paragraph 00115 of the originally filed specification, and noting that no actual written description of H784A mutant polymerase is provided).

12. Claims 1, 5, 9-12, 14-17, 19-20, 77-79, 81-85, 88, 90-94, 101-103, 108-110, 112-114, 116-120, 122-127, 130-137, 139-148, 150-156, 159, 159, 161-174, 176-190, 195 and 196 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The current claims recite methods for identifying apatmers, comprising preparing single stranded nucleic with at least one 2'-OMe GTP being incorporate by a modified RNA polymerases with at least one mutated amino acid residue, wherein the

composition further comprises transcription reaction components. Further the dependent claims recite various modifications of the reaction conditions and components. This large genus of modified RNA polymerases is represented in the specification by the one modified RNA polymerase which is Y639F/H784A double mutant T7 RNA polymerase. Thus, applicant has expressed possession of only one species in a genus, which comprises hundreds of millions of different possibilities, owing to the multitude of mutants possible for the plethora of RNA polymerases.

What the specification teaches: (1) only one modified RNA polymerase functions as claimed which is the Y639F/H784A double mutant T7 RNA polymerase, (2) that the Y693F single mutant polymerase does not function as claimed, and (3) fails to provide evidence that the other disclosed modified RNA polymerase which is the H784A single mutant functions as claimed. The specific disclosure is:

"Generally, it has been found that under the conditions disclosed herein, the Y693F [sic, Y639F] single mutant can be used for the incorporation of all 2'-OMe substituted NTPs except GTP and the Y639F/H784A double mutant can be used for the incorporation of all 2'-OMe substituted NTPs including GTP. It is expected that the H784A single mutant possesses similar properties when used under the conditions disclosed herein" (see paragraph 00115 of the originally filed specification).

The specification does not show any specific modified RNA polymerases other than the three given above.

What is reduced to practice: only the Y639F/H784A double mutant T7 RNA polymerase is reduced to practice.

What the drawings show: the drawings do not show any modified RNA polymerase functioning as claimed.

The written description guidelines note regarding such genus/species situations that "Satisfactory disclosure of a "representative number" depends on whether one of skill in the art would recognize that the applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of the species disclosed." (See: Federal Register: December 21, 1999 (Volume 64, Number 244), revised guidelines for written description.) The essential identifying characteristic of the claimed modified RAN polymerases is that they function to incorporate 2-O-methyl GTP. Here, no common elements and attributes of the mutants of the RNA polymerases as set forth in the independent claims 1, 101, 102, and 182 are disclosed for this function. With regard to the various mutations of the RNA polymerases with various combinations of different mutated amino acid residues, this is insufficient to demonstrate identity of all specific modified RNA polymerases of the claimed invention. Instant claims are overly broad in the recitation of "comprising" since no guidance is provided as to which of the variant mutant RNA polymerases would function to incorporate 2'-OMe GTP into a single stranded nucleic acid. Further no information is given in the specification regarding a methodology to determine such common elements or attributes.

To fulfill the written description requirement, a patent specification must describe an invention and do so in sufficient detail that one skilled in the art can clearly conclude that "the inventor invented the claimed invention." *Lockwood v. American Airlines, Inc.*, 107 F.3d 1565, 1572, 41 USPQ2d 1961, 1966 (1997); In *re Gosteli*, 872 F.2d 1008, 1012, 10 USPQ2d 1614, 1618 (Fed. Cir. 1989) (" [T]he description must clearly allow persons of ordinary skill in the art to recognize that [the inventor] invented what is claimed."). Thus, an applicant complies with the written description requirement "by describing the invention, with all its claimed limitations, not that which makes it obvious," and by using "such descriptive means as words, structures, figures, diagrams, formulas, etc., that set forth the claimed invention." *Lockwood*, 107 F.3d at 1572, 41 USPQ2d at 1966.

It is noted that in Fiers v. Sugano (25 USPQ2d, 1601), the Fed. Cir. concluded that "...if inventor is unable to envision detailed chemical structure, as well as method of obtaining it, then conception is not achieved until reduction to practice has occurred, that is, until after the compound has been isolated. In the application at the time of filing, there is no record or description which would demonstrate conception or written description of various mutant RNA polymerases modified by mutations including insertions and deletions of amino acids and no correlative protein structure claimed.

Predictability of the art: the unpredictability in the art is high as evidenced by Chelliserrykattil and Ellington (2004 cited on the IDS and by Applicant in Remarks filed 06/11/2009) who teach three modified RNA polymerases would not function for incorporation of 2' O-methyl GTP (see last sentence in the 1st column continued to the end column on p. 1157). Thus the level of skill in the art is deemed to be high.

And, while one of ordinary skill would have been able to perform the methods with the Y639F/H784A double mutant T7 RNA polymerase, applicant clearly has not shown that any other modified RNA polymerase would function as claimed. Accordingly, the specification does not provide a written description of the invention of claims 1, 5, 9-12, 14-17, 19-20, 77-79, 81-85, 88, 90-94, 101-103, 108-110, 112-114, 116-120, 122-127, 130-137, 139-148, 150-156, 159, 159, 161-174, 176-190, 195 and 196.

New Claim Rejections - 35 USC § 103

13. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

14. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

15. Claims 1, 5-7, 9-12, 14-16, 19-20, 77-79, 81-85, 88, 90-94, 101-105, 107-109, 112-114, 116-120, 122-127, 133-136, 139-147, 150-155, 158, 161-173, and 176-190 are rejected under 35 U.S.C. 103(a) as being unpatentable over by Pieken et al. (U.S. Patent 5,660,985 previously cited), Cook et al. (United States Patent 5,914,39 issued 1999), Brieba et al. (Biochemistry (2000) 39:919-923 previously cited), Sousa et al (U.S. Patent 6,107,037 previously cited), Bishop et al. (1971, previously cited), and Chow et al. (1971).

Pieken teaches methods of claims 1, 101, 102, 145, 156, 167, 181, 182, 184, and 186 for identifying nucleic acid ligands that bind to a target molecule (see abstract) wherein the nucleic acid ligands comprise a 2'-OMe modified nucleotide (see claim 1 and claim 10, where 2' methoxy groups are expressly claimed),
(a) preparing a transcription mixture comprising a polymerase, modified dNTPs, wherein at least one NTP is 2' OMe NTP where N can be A, G, C, T or U (by teaching modified pyrimidine and purine bases can be 5-X and/or 2'-Y, here being 2'-Y only with Y being the methoxy group, see column 8 lines 38-63 and Figure 1), and specifically can be 2'-OMe guanosine (see the bottom left structure in Figure 1 and without the X substitution as provided for in column 1 line 25), magnesium and oligonucleotide transcription templates (see column 16, example 3, lines 10-1 3, where GTP, which is a 2'-OH guanosine triphosphate is used and see claim 10, which requires the use of a 2' OMe NTP),

(b) preparing a candidate mixture of single-stranded nucleic acids by transcribing the one or more oligonucleotide transcription templates including double standed

templates (see Example 2) under conditions whereby the polymerase incorporates at least one of the one or more 2' O-methyl modified NTPs into nucleic acid molecules of said candidate mixture (see column 16, lines 13-35, where the T7 RNA polymerase is used to incorporate the NTPs and see claim 10, where the modified nucleotides are 2' O-methyl modified NTPs) wherein the stabilized single-stranded nucleic acids have a length in the range of 30-50 nucleotides by teaching a 2'-O-methyl stabilized oligonucleotide of similar length to a 2'-amino oliognucleotide of 38 nucleotides (see Example 6),

(c) contacting the candidate mixture with said target molecule (see column 16, example 3, lines 13-35 and claim 1),

(d) partitioning the nucleic acids having an increased affinity to the target molecule relative to the candidate mixture from the remainder of the candidate mixture (see column 16, example 3, lines 13-35 and claim 1),

(e) amplifying the increased affinity stabilized nucleic acids which are oligonuceltoides, in vitro, to yield a ligand enriched mixture of nucleic acids, whereby nucleic acid ligands of the target molecule are identified (see column 16, example 3, lines 13-35 and claim 1, and see column 20 lines 14-17 for the stabilized oligonucleotide).

Further regarding claims 1, 101, 102, and 182, Pieken teaches the use of 2' OH-guanosine which is a substituted guanosine (see column 16, example 3, lines 10-13, where GTP, which is a 2'-OH guanosine triphosphate is used).

With regard to claims 9-11, 90, 91, 107, 108, 109, 122, 123, 124, 139, 140, 150, 151, 161, 162, 168, 169, and 170, Pieken teaches a purine leader sequence which is 6 nucleotides in length (see SEQ ID NO: 3). Pieken also teaches the use of 2' OH-guanosine which is a substituted guanosine (see column 16, example 3, lines 10-13, where GTP, which is a 2'-OH guanosine triphosphate is used).

With regard to claims 19-20, 85, 94, 112-113, 127, 143, 154, 165, 176, and 177 Pieken teaches the use of PEG (see column 15, line 49).

With regard to claims 77, 83, 92, 116-119, 125, 141, 152, 163, and 178, Pieken teaches a variety of ratios and mixtures of modified to unmodified nucleotides (see column 13, lines 5-7).

With regard to claims 78, 84, 93, 126, 142, 153, 164, and 179, Pieken teaches the transcription mixture can further comprise spermidine (see Example 2).

With regard to claims 81, 82, 90, 91, 107-109, 122-124, Pieken teaches a purine leader sequence which is 6 nucleotides in length (see SEQ ID NO: 3).

With regard to claims 188 and 190, Pieken teaches repeating the claim steps (see claim 1).

Regarding claims 1, 101, 102, and 182, Pieken does not specifically teach the use of modified polymerase and does not teach the use of Y639F or H784A T7 RNA polymerase. Pieken does not specifically teach the use of manganese and does not specifically teach the single stranded nucleic acids.

Regarding claims 1, 5-7, 101-105, 116-119, 134, 135, 146, and 182 Brieba teaches that T7 polymerase mutants at position 784 preferentially utilize 2'-OH groups (see abstract) and position 639 mutants rapidly incorporate 2' modified nucleotides (see page 920). Brieba does not specifically teach the use of manganese and does not specifically teach the single stranded nucleic acids in the range of 30-50 nucleotides with incorporated 2'-O-methylribobnucletides.

Regarding claims 1, 14-16, 101, 102, 116-119, 133, 144, 155, 166, 171-173, 180, 182, 183, 185, 187, and 188, Sousa also teaches the use of manganese and magnesium (see column 15, lines 44-48) but does not specifically teach the combination of manganese and magnesium ions. Sousa does not specifically teach the single stranded nucleic acids in the range of 30-50 nucleotides with incorporated 2'-O-methylribobnucletides.

Although this is new rejection, it is noted that Applicant argues that Sousa teaches away from manganese. However, Sousa teaches the use of manganese as given above, and thus does not teach away from manganese. Furthermore, it is the combination of manganese and magnesium which the claims recite and Sousa does not teach away from this combination. Additionally, in light of the teachings of Bishop et al. (below) one of ordinary skill in the art would have been motivated to combine manganese and magnesium as this combination is superior to either manganese or magnesium alone.

Regarding claims 12, 79, 88, 114, 120, 136, 147, and 158, Sousa teaches: "Preferably, the reactions also contain inorganic pyrophosphatase, which is known to increase the yields in *in vitro* transcription reactions" (see column 12 lines 41-43).

Regarding claims 1, 14-16, 101, 102, 116-119, 133, 144, 155, 166, 171-173, 180, 182, 183, 185, 187, and 188, Bishop et al. teach the use of combined manganese and magnesium for optimum performance of RNA polymerases in transcription by teaching: "The optimal conditions for assaying influenza (WSN) virion [ribonucleic acid] polymerase have been determined. The enzyme is maximally active . . . in reactions containing . . . 1 to 2 mm $MnCl_2$, . . . 8 to 10 mm $MgCl_2$, and the four ribonucleoside triphosphates at levels above certain delineated threshold values" (entire article, especially the first two sentences under the Discussion section on p. 69 and Figures 2 and 3) and by teaching in vitro synthesis/transcription with RNA polymerase (see Title, Abstract, and first sentence on p. 66). Furthermore, Bishop et al. teach concentration ratios of magnesium ions to manganese ions of 4 to 10 (8 mm $MgCl_2$ / 2 mm $MnCl_2$, = 4 and 10 mm $MgCl_2$ / 1 mm $MnCl_2$, = 10) which overlaps the claimed concentration ratios of magnesium ions to manganese ions of about 3 to 5. Bishop does not specifically teach the single stranded nucleic acids in the range of 30-50 nucleotides with incorporated 2'-O-methylribobnucletides.

Although this is a new rejection, it is noted that Applicant argues that Bishop does not teach a modified polymerase (relative to the WSN virion polymerase). However, Bishop teaches virion polymerases and thus teaches at least a type of

unmodified polymerase as recited in the instant claims and further teaches the use of a unmodified WSN virion polymerase with the combination of manganese and magnesium and teaches how to go about optimizing this and other polymerase conditions including incorporation of triphosphate nucleotides for maximal synthesis. Thus it would have been obvious to one of ordinary skill in the art to use the optimization approach of Bishop for other polymerases, especially modified WSN virion polymerases. This is further evidenced by Chow et al., as given following.

Regarding claims 1, 14-16, 101, 102, 116-119, 133, 144, 155, 166, 171-173, 180, 182, 183, 185, 187, and 188, Chow et al. teach the use of combined manganese and magnesium for optimum performance of RNA polymerases from nine virus strains including stains of Influenza A and B and one of which is from the WSN strain as taught by Bishop (see Table 2) in transcription, by teaching that *in vitro* RNA synthesis of each strain had an obligate requirement for Mn2+ (see Abstract) and that optimal performance was achieved in the presence of both 0.38 umol $MnCl_2$ and 1 umol $MgCl_2$ (which is 2.6 or about 3 times greater magnesium ions than manganese ions, entire article especially the complete assay results in Table 1 and see also Figure 1). Furthermore, Chow teaches 25 mM $MgCl_2$ and 5 mM $MnCl_2$ (see Figure 1 B) which is 5 times greater magnesium ions than manganese ions. In addition, Chow teaches that that RNA polymerase activity in the nine strains varies due to genetic recombination (see last paragraph on p. 756). In other words, an RNA polymerase of any one strain is a modified polymerase of any the other eight remaining strains and which modified RNA

polymerase reads on the instant claims, as at least one amino acid residue must necessarily be mutated in the modified RNA polymerases. Using the polymerase from the WSN virion strain as the unmodified polymerase, the other eight polymerases are the modified polymerases of at least instant claims 1, 101, and 102 and noting that the modified polymerase which is from the WS strain gives a greater incorporation rate of triphosphate nucleotides than the unmodified polymerase from the WSN strain (see Table 2). And Chow et al. also teach how to identify the modified polymerase with the most optimal ability to incorporate triphosphate nucleotides specifically by varying amounts of both magnesium ions and manganese ions (entire publication, especially Table 2). Chow et al. do not specifically teach the single stranded nucleic acids in the range of 30-50 nucleotides with incorporated 2'-O-methylribobnucletides.

Thus, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to use the T7 RNA polymerase mutants of Brieba in the methods of Pieken since Brieba notes that the polymerase with the double mutant is more likely to incorporate 2' O substituents (see abstract), and since Pieken would be motivated by this teaching to utilize the modified polymerase with superior properties for incorporation of the desired 2' modified nucleotides, including in the presence of both magnesium ions and manganese ions as taught by Bishop et al. and Chow et al. for incorporation of nucleotides.

It would have been prima facie obvious to one of ordinary skill in the art at the

time the invention was made to use the magnesium/manganese buffers of Sousa, Huang et al., Bishop et al., and Chow et al. in the methods of Pieken, and Brieba. Sousa teaches regarding the use of manganese that "In Mn buffer both the w.t. enzyme and Y639F show a reduction in their sensitivity to substitution of dNTPs for rNTPs, consistent with an expectation of reduced substrate discrimination in Mn buffer (see column 22, lines 34-37)" and to use: ". . . inorganic pyrophosphatase . . . to increase the yields in in vitro transcription reactions" (see column 12 lines 41-43). Both Bishop et al. and Chow et al. teach the combined use of magnesium and manganese ions within the claimed ratio range to achieve optimum performance of in vitro transcription by RNA polymerases. Chow further teaches that this optimum performance is achieved with modified RNA polymerases. Thus an ordinary practitioner would have been motivated to use manganese ions and magnesium ions in optimized concentrations for modified RNA polymerase in order to permit incorporation of the modified nucleotides expressly desired by Pieken and Brieba. And thus, the claimed invention as a whole was *prima facie* obvious over the combined teachings of the prior art.

16. Claims 17, 110, 130-132, 137, 148, 159, and 174 are rejected under 35 U.S.C. 103(a) as being unpatentable over Pieken et al. (U.S. Patent 5,660,985), Brieba et al. (2000), Sousa et al (U.S. Patent 6,107,037), Bishop et al. (1971), and Chow et al. (1971) in view of Milligan et al. (Methods Enzymol. (1989) previously cited).

Pieken, Brieba, Sousa, Bishop, and Chow teach as noted above.

Pieken, Brieba, Sousa, Bishop, and Chow do not teach the use of GMP in T7 RNA polymerase reactions.

Milligan teaches that when "modified GTP is to be used, it is a good idea to add GMP as a primer if low concentrations of GTP are to be used (see page 59)."

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to use GMP as taught by Milligan when performing the SELEX method of Pieken, Brieba, Sousa, Bishop, and Chow using modified GTP such as 2'-0 methyl GTP since Milligan states that when "modified GTP is to be used, it is a good idea to add GMP as a primer if low concentrations of GTP are to be used (see page 59)." An ordinary practitioner would have been motivated to add GMP whenever low GTP amounts or modified GTP is being used in transcription reactions, in order to ensure the ability of the T7 RNA polymerase enzyme to prime the extension reaction.

17. Claims 8, 106, 157, 195, and 196 are rejected under 35 U.S.C. 103(a) as being unpatentable over Pieken et al. (U.S. Patent 5,660,985), Brieba et al. (2000), Sousa et al (U.S. Patent 6,107,037), Bishop et al. (1971), and Chow et al. (1971) as applied to claims 5, 103, and 134 above and in further view of Padilla et al. (2002 published December 15, 2002).

Pieken, Brieba, Sousa, Bishop, and Chow teach as noted above.

Pieken, Brieba, Sousa, Bishop, and Chow do not specifically teach the Y639F/H784A T7 RNA polymerase double mutant T7 RNA polymerase.

Regarding claims 8, 106, and 157, Padilla et al. teach the Y639F/H784A T7 RNA polymerase double mutant T7 RNA polymerase for incorporating 2'-OMe NTPs into nucleic acids (entire article, especiallly p. 2 and Table 1) and thus generally but do not specifically teach 2'-OMe GTPs.

Regarding claims 195 and 196, Padilla et al. teach the Y639F/H784A T7 RNA polymerase double mutant T7 RNA polymerase for incorporating 2'-OMe NTPs and NTPs into nucleic acids (entire article, especiallly p. 2 and Table 1) and teach that this can be done without premature termination products and thus generally teach stabilized apatmers can be prepared with any percentage of 2'-OMe NTPs and NTPs including 80% of which are 2'OMe GTP and the remaining are 2'OH GTP but do not specifically teach 2'OMe GTP and 2'OH GTP.

Therefore, it would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to modify the methods of Pieken, Brieba, Sousa, Bishop, and Chow by using the Y639F/H784A T7 RNA polymerase double mutant T7 RNA polymerase for incorporating 2'-OMe NTPs into nucleic acids as suggested by Padilla et al. with a reasonable expectation of success. The motivation to do so is provided by Padilla et al. who teach the Y639F/H784A T7 RNA polymerase double mutant T7 RNA polymerase has: " . . . an enhanced ability to incorporate NMPs with bulky 2'-substituents into RNA. In reactions with 2'-OMe- or 2'-azido-modified NTPs yields of run-off transcripts, relative to reactions with the four canonical NTPs, are markedly increased with the double mutant and premature termination products are

greatly reduced or eliminated" (see 1st sentence of the *Discussion* section on p. 3). Thus, the claimed invention as a whole was *prima facie* obvious over the combined teachings of the prior art.

Conclusion

18. No claim is allowed.

19. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Mark Staples whose telephone number is (571) 272-9053. The examiner can normally be reached on Monday through Thursday, 9:00 a.m. to 6:00 p.m.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Mark Staples/
Examiner
Art Unit 1637
August 21, 2009